

**CLAIMS:**

1. A method for repetitive DNA expression cassette exchange in the genome of cells or parts of cells comprising the steps of

- a) integrating into a chromosomal locus of the genome of said cells a first DNA expression cassette carrying a positive-negative selection marker flanked by a wild type FLP-recombinase recognition target (FRT) site on one end and a modified heterospecific FRT site on the other end for tagging,
- b) selecting cell clones surviving the conditions for positive selection,
- c) exchanging said first DNA expression cassette against an incoming second DNA expression cassette located on a circular vector and carrying a homologous or heterologous gene (transgene) of any coding sequence flanked by the same FRT sites as said first DNA expression cassette mediated by the action of FLP-recombinase,

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said cells are vertebrate cells which can regenerate to

complete organisms, and said parts of cells are nuclei of vertebrate cells, which can be inserted into regenerative cells,

and further characterized by

- d) maintaining the conditions for positive selection during cultivation of said cells obtained in step b) while exchanging said first DNA expression cassette against said incoming second DNA expression cassette,
  - e) using in step c) an incoming second DNA expression cassette which is marker-free, and
  - f) selecting cell clones obtained after step c) surviving the conditions for negative selection.
2. The method according to claim 1 wherein said positive-negative selection marker is a hygromycin-B-phosphotransferase and HSV-thymidine kinase encoding (hyg<sup>tk</sup>) fusion gene.
  3. The method according to claim 1 or 2 wherein said modified heterospecific FRT site is a FRT spacer mutant.
  4. The method according to claim 3 wherein said FRT spacer mutant is the F<sub>3</sub> mutant.

5. The method according to any of the preceding claims wherein said regenerative vertebrate cells are vertebrate embryonic stem (ES) cells.
6. The method according to claim 5 wherein said vertebrate embryonic stem cells are mouse embryonic stem cells.
7. Regenerative vertebrate cells comprising a modified genome obtainable by a method according to any one of claims 1 to 6.
8. Nuclei of vertebrate cells comprising a modified genome obtainable by a method according to any one of claims 1 to 6.
9. Regenerative vertebrate cells containing a nucleus according to claim 8.
10. A method for generation of transgenic vertebrates characterized by injecting regenerative vertebrate cells according to claim 7 or 9 into blastocysts of said vertebrate.
11. A method according to claim 10, characterized in that said vertebrates are mice.